

UNCLASSIFIED

AD 290 509

*Reproduced
by the*

**ARMED SERVICES TECHNICAL INFORMATION AGENCY
ARLINGTON HALL STATION
ARLINGTON 12, VIRGINIA**



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

63-1-5

AMRL-TDR-62-110

290509

**STUDY OF THE INTERMEDIARY METABOLIC
PATHWAYS OF
1,1-DIMETHYLHYDRAZINE (UDMH)**

TECHNICAL DOCUMENTARY REPORT No. AMRL-TDR-62-110

SEPTEMBER 1962

Biomedical Laboratory
6570th Aerospace Medical Research Laboratories
Aerospace Medical Division
Air Force Systems Command
Wright-Patterson Air Force Base, Ohio

Contract Monitor: Dr. Kenneth C. Back
Project No. 6302, Task No. 630202

[Prepared under Contract No. AF 33(616)-8455

by
M. A. Mitz
F. L. Aldrich
B. M. Vasta
Melpar, Inc., Falls Church, Virginia]

ASTIA

CATALOGED BY

AS 60110

290 509

ASTIA
DEC 10 1962
TISIA

FOREWORD

This study was initiated by the Biomedical Laboratory of the 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio, under Contract No. AF 33(616)-8455. Dr. Milton A. Mitz, Head, Life Sciences Section, was the principal investigator for Melpar, Inc. Dr. Kenneth C. Back of the Toxic Hazards Section, Physiology Branch, was the contract monitor from the 6570th Aerospace Medical Research Laboratories. The work was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630202, "Pharmacology and Biochemistry," in the Life Sciences Section, Melpar, Inc. The research sponsored by this contract was started in July 1961 and was completed in June 1962. The experiments reported were conducted according to the "Rules Regarding Animal Care" established by the American Medical Association.

ABSTRACT

The metabolic fate of 1,1-dimethylhydrazine (UDMH) was studied in female rats and dogs. The animals were given intraperitoneal injections of C^{14} labelled UDMH and, after an appropriate time, body tissues were examined by paper chromatography. Six compounds were found, although not all appeared in every tissue. Three major compounds were present in urine. Identification showed one (3-10%) to be the glucose hydrazone of UDMH, and another appeared to be unreacted UDMH (50-60%). The structure of the third major component (20-25%) was not determined, although its chemical and chromatographic behavior suggested that it was a hydrazone or hydrazide of a compound of higher molecular weight than acetic or pyruvic acid. Various analytical procedures were employed for the determination of UDMH in urine, and the trisodium pentacyanoaminoferroate (TPF) procedure was found to be the most sensitive and most specific qualitative method of those tested.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

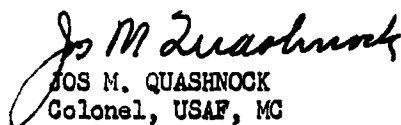

JOS M. QUASHNOCK
Colonel, USAF, MC
Chief, Biomedical Laboratory

TABLE OF CONTENTS

	Page
I INTRODUCTION	1
II MATERIALS AND METHODS	1
III RESULTS	3
A. DETECTION AND DETERMINATION OF UDMH	3
1. Selection of Reagent	3
2. Inhibitor of the TPF Reaction	3
3. Selective Destruction or Binding of UDMH	6
B. IDENTIFICATION OF METABOLITES	9
1. Distribution and Balance Studies	9
2. Respiration Studies	10
3. Chromatographic Examination of Tissues	11
4. Large-Scale Fractionation Studies	13
5. Synthesis of Possible Metabolites	15
6. Behavior of Synthetic Compounds	17
IV DISCUSSION	19
V SUMMARY	21
VI REFERENCES	22

I. INTRODUCTION

The compound 1,1-dimethylhydrazine (unsymmetrical dimethylhydrazine, UDMH), in common with hydrazine itself and other derivatives, serves as a high-energy fuel. The toxicity of compounds of this class has long been recognized, and it constitutes a health hazard both for personnel using them at military establishments and for civilian workers at sites of manufacture. The routes of administration appear to have little effect on the toxicities.

McKennis et al. (refs. 1,2,3) have studied the metabolic fate of hydrazine and certain physiologically significant derivatives. Their reports showed that while diacetylation occurs in man and rabbits, only monoacetylation occurs in dogs. Possible metabolic products other than acetyl compounds were not investigated.

In the case of UDMH, it is unlikely that diacetylation could occur in any species. Acylation of a nitrogen atom that already possesses one acyl substituent is a very difficult process, and acylation of the dimethyl-substituted nitrogen atom could not occur without prior or simultaneous demethylation.

The prospects for finding effective therapeutic measures would be greatly improved if the fate of UDMH were known. Thus, if it were known that, biologically, a particular compound reacts with and binds UDMH, that substance, if not toxic itself, could be used to overcome the effects of UDMH-induced convulsions in monkeys (ref. 4). The in vivo formation of the dimethylhydrazine of pyridoxal-5-phosphate is considered a probable cause of interference with some pyridoxal-requiring enzymes of the nervous system. Therefore, definite knowledge of the nature of the metabolites of UDMH is very desirable. The research described subsequently was designed to provide this desired information.

II. MATERIALS AND METHODS

Experimental Animals

Rats and dogs were subjected to doses of UDMH by intraperitoneal (i.p.) injection. Urine was collected in metabolic cages. All animals used were given water ad libitum, but food was not provided during experiments. Animals used for tissue studies were killed by decapitation (rats) or electrocution (dogs).

Materials

1,1-dimethylhydrazine ("Dimazine") was obtained from the Chlor-alkali Division, Food Machinery and Chemical Corporation, New York, New York.

C^{14} -labelled UDMH was synthesized by and obtained from Tracerlab Inc., Waltham, Massachusetts. This material had a specific activity of 5.74×10^{-2} mc/mM. UDMH with a specific activity of 1.90×10^{-3} mc/mM (25 μ c/ml) was obtained through the courtesy of Dr. K.C. Back, Toxic Hazards Section, Biomedical Branch, Wright-Patterson Air Force Base. The C^{14} -UDMH was diluted with 0.23 M citric acid, divided into aliquots in ampules which were then sealed, and stored in a freezer. For use, the contents were thawed, the ampule was opened, and a basic solution (0.5-1.0 M Na_2HPO_4) was added to produce the desired final concentration and pH. This procedure avoided the gradual decomposition of UDMH encountered in the absence of such precautions.

PPO (2,5-diphenyloxazole) and POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] were obtained from the Packard Instrument Company, La Grange, Illinois.

Trisodium pentacyanoaminoferroate (TPF) was a Fisher Chemical Company product, batch No. 782190. Some preparations of this compound were found to produce little color upon reaction with UDMH, so all the work was done with a single batch found to be satisfactory.

All other chemicals used were highest-quality commercial products.

Chromatography

All chromatography was conducted using the descending technique on 1" x 22 1/2" strips of Whatman No. 1 paper. Sizes of aliquots were chosen to keep the amount of UDMH below 10 micrograms. No pH adjustment was made on biological samples because of the danger of altering equilibria between free and bound UDMH.

Preparation of Tissues for Radioactivity Measurements

The animals were killed as described above, the organs were immediately removed, trimmed of excess fat, and the wet weight determined. Representative portions of each organ weighing 0.5-1.0 gram were homogenized in the cold with 2 volumes of 1.15% aqueous KCl solution. Aliquots (0.01-0.10 ml) were placed in counting vials, and 20 ml of scintillation liquid was added. Aliquots of blood and urine were placed directly in vials with scintillation liquid. The vials were shaken briskly to obtain a homogeneous liquid and then counted. The scintillation liquid was prepared by dissolving 4.0 g of PPO and 0.1 g of POPOP in 1,000 ml of an 80:20 mixture by volume of toluene and 95% ethanol.

Preparation of Chromatograms for Radioactivity Measurements

The dried, developed chromatogram strips (1" x 22 1/2") were cut into small strips (0.4" x 1"), and each such small strip was added and the vial was shaken and counted. Orientation of the strips in the vials was experimentally determined to have no effect on count rates.

C¹⁴ Counting Techniques

All counting was done in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Company). Tissue samples were counted for periods of time sufficient to give a standard deviation no greater than +10%. Chromatogram strips were counted 3-5 minutes, and the standard deviation varied from +5% to +20%, depending on the count rate. Backgrounds were determined using tissues from control animals or strips from control chromatograms. The counting efficiency of the instrument was found to be 50-60 % using control samples of the C¹⁴-UDMH. Quenching factors were determined for each sample by adding 1 ml of a 1 to 100,000 dilution of the low specific activity C¹⁴-UDMH in scintillation liquid and repeating the counts. Counts per minute (cpm) were converted to disintegrations per minute (dpm) using the quench factor for each sample, and these activities were equated to micrograms of UDMH.

III. RESULTS

A. DETECTION AND DETERMINATION OF UDMH

1. Selection of Reagent

An investigation was conducted of reagents and methods which have been proposed for the detection and/or determination of UDMH or other hydrazines. Those included were ammoniacal silver nitrate (ref. 5), p-dimethylaminobenzaldehyde (DAB) (ref. 6), phosphomolybdic acid (PMA) (ref. 7), pyridoxal phosphate (PALP), and trisodium pentacyanoaminoferroate (TPF) (ref. 8). Although the first three reagents mentioned are lacking in specificity, two of them (DAB and PMA) were selected as spray reagents for the chromatographic studies described later. The PALP reagent was found to be less sensitive for UDMH than TPF, so the latter was adopted as the reagent of choice for determining UDMH in urine. TPF was also used as a chromatographic spray reagent.

A published method for measuring UDMH in air, water, and blood (ref. 9) was modified for our use as follows: A 1-5 ml sample was mixed with enough citrate-phosphate buffer (0.023 M citrate, 0.055 M phosphate, pH 5.4) to make a total volume of 10.0 ml. Next, 1.0 ml of a 0.1% aqueous solution of TPF was added and the reaction was allowed to proceed for 60 minutes. The optical density was measured at 500 mμ against a reagent blank set to zero optical density. However, the fact that many samples of urine contain materials which interfere with the development of a full color poses a serious disadvantage. Consequently, attention was directed toward determining the nature and overcoming the effects of the urinary inhibitors(s).

2. Inhibitor of TPF Reaction

Preliminary experiments were conducted to determine whether or not the interfering materials were ions. Aliquots of an inhibitory urine were treated with calcium chloride (0.01 M), aluminum chloride (0.01 M), ethylenediaminetetraacetic acid (EDTA, 0.01 M) and mercuric chloride (3×10^{-4} M). The last-named compound caused a further decrease in color formation by reacting with TPF, but the failure of EDTA to overcome the normal inhibition ruled out metal ions as the factor responsible for low values. Anions which form insoluble calcium salts, such as oxalate, sulfate, and carbonate, were likewise eliminated from consideration by these experiments. Thiols, as well as thiosulfate and thiocyanate ions, were eliminated by conducting UDMH analyses with TPF on samples treated with silver nitrate. The precipitate obtained was removed, and the excess silver ion was precipitated by adding KCl. The filtrate from the AgCl was employed for analysis, and it gave the same result as the untreated urine.

Next, the effect of concentration of UDMH in urine on the degree of inhibition was investigated. A sample of urine known to exhibit inhibition was selected, and various known amounts of UDMH were added to aliquots of the urine. The UDMH was determined by the TPF procedure previously described. Aqueous standards were similarly analyzed. The results (figure 1) suggested that the inhibitory substance in urine exerted its influence by reacting with the TPF reagent, rendering the TPF concentration insufficient for full color development.

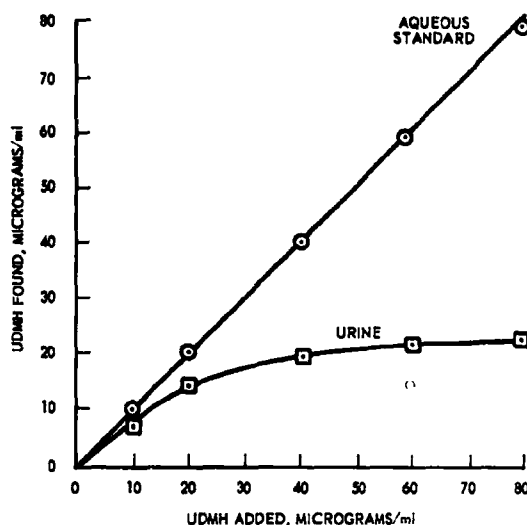


Figure 1. Effect of UDMH Concentration on Recovery in Urine

The possibility that inhibition might be overcome by using more TPF was investigated by conducting analyses with four concentrations of TPF. Solutions of UDMH (40 µg/ml) in water and in an inhibitory urine were prepared. Aliquots of each were analyzed in the manner previously described using various concentrations of the TPF reagent. The results (table 1) supported the conjecture that a TPF concentration of 0.1% was insufficient in the presence of urine and suggested that a concentration of at least 0.2% should be used.

TABLE 1

EFFECT OF TPF CONCENTRATION ON UDMH RECOVERY

Concentration of TPF reagent	Optical density at 500 mμ		Percent of standard
	Aqueous standard	Urine + UDMH	
0.1%	0.499	0.203	40
0.2%	0.477	0.472	99
0.5%	0.524	0.537	103
1.0%	0.526	0.589	110

To demonstrate the usefulness of high TPF concentrations for quantitative determination of UDMH in urine, four specimens of human urine were used. UDMH (20 µg/ml) was added to each and 1.0 ml aliquots were analyzed using both 0.1% and 0.5% TPF. For the latter reagent concentration a reaction time of 15 minutes was used because the maximum color was attained in this shorter time under these conditions. An aqueous standard of 20 µg/ml was similarly analyzed. The data (table 2) showed that a recovery of at least 91% of the added UDMH was obtained in all cases using the 0.5% reagent while the best recovery using the 0.1% reagent was only 59%.

TABLE 2

EFFECT OF HIGH TPF CONCENTRATION ON UDMH RECOVERIES

<u>Sample</u>	<u>Observed UDMH concentration, $\mu\text{g/ml}$</u>		<u>Percent Inhibition</u>	
	<u>0.1% TPF</u>	<u>0.5% TPF</u>	<u>0.1%</u>	<u>0.5%</u>
Aqueous standard	20.0	20.0	-	-
Urine 1	5.1	19.1	74.5	4.5
Urine 2	10.6	18.2	47.0	9.0
Urine 3	9.2	19.0	54.0	5.0
Urine 4	11.8	18.8	41.0	6.0

During the study described immediately above, it was noted that the optical density of a mixture of urine, buffer, and TPF reagent was greater than the sum of the optical densities of urine plus buffer and buffer plus TPF. The possibility that the urinary constituent responsible for this phenomenon was the source of "inhibition" of color formation was investigated. UDMH (20 $\mu\text{g/ml}$) was added to a portion of each of 9 specimens of human urine. The reaction mixtures described below were prepared and, after a 60-minute reaction period, the optical density of each was measured at 500 $\text{m}\mu$ against distilled water.

- 1.0 ml 20 $\mu\text{g/ml}$ aqueous UDMH standard, 9.0 ml buffer, 1.0 ml 0.1% TPF.
- 1.0 ml water, 9.0 ml buffer, 1.0 ml 0.1% TPF.
- 1.0 ml urine containing 20 $\mu\text{g/ml}$ UDMH, 9.0 ml buffer, 1.0 ml 0.1% TPF.
- 1.0 ml urine without UDMH, 9.0 ml buffer, 1.0 ml 0.1% TPF.
- 1.0 ml urine without UDMH, 9.0 ml buffer 1.0 ml water.

The degree of inhibition was calculated for each urine specimen by the relation:

$$\text{Percent Inhibition} = \frac{(A-B) - (C-D)}{A-B} \times 100, \quad (1)$$

the terms (A-B) and (C-D) being the optical densities of the standard and sample, respectively, when measured against the corresponding blanks. The "apparent" UDMH (the concentration of UDMH in water that would produce an identical result) of urine containing no UDMH was calculated as

$$\mu\text{g/ml UDMH equivalent} = \frac{D-(B+E)}{(A-B)} \times 20 \quad (2)$$

The results (table 3) showed a lack of correlation between the two values calculated. The normal urinary constituent that produces optical density at 500 $\text{m}\mu$ upon reaction with TPF was, therefore, shown to be only one source of the apparent inhibition.

These data indicated that other inhibitory urine constituents react with TPF without producing optical density at 500 $\text{m}\mu$. A brief study of spectral changes occurring during the reaction of TPF with urine revealed an increase in optical density at 394 $\text{m}\mu$, presumably because of these other constituents.

TABLE 3

LACK OF CORRELATION BETWEEN INHIBITION AND TPF REACTION

<u>Sample No.</u>	<u>Percent inhibition</u>	<u>Apparent UDMH in urine, $\mu\text{g/ml}$</u>
1	88	3.7
2	79	5.5
3	76	4.0
4	75	0.2
5	55	0.1
6	50	3.5
7	44	3.3
8	37	4.0
9	32	4.6

The use of high concentrations of TPF will largely overcome the effects of inhibitors in recovery experiments such as those described above. However, the presence of normal urinary constituents that give rise to color at 500 m μ with TPF renders such a procedure useless for real samples. No appropriate blank urine, identical with the sample except for UDMH, would be available, so erroneous results would be obtained. If the UDMH of a sample could be destroyed or bound by a reaction without affecting the inhibitors, a suitable blank would result. The possibility of achieving a selective destruction or binding of UDMH in urine was the subject of the next phase of this study.

3. Selective Destruction or Binding of UDMH

Hydrazines react readily with carbonyl compounds to form hydrazones or hydroxymethylhydrazines, and hydrazones are also readily oxidized by oxidizing agents. One compound of each class of reagents was selected for study, formaldehyde as a carbonyl compound and iodine as an oxidizing agent.

a. Formaldehyde (HCHO)

Preliminary experiments showed that UDMH in aqueous solution was destroyed (or bound) by less than 4% HCHO. Solutions resulting from this treatment behaved exactly like a water blank on addition of TPF. The usefulness of HCHO for overcoming the effects of inhibition was then tested. UDMH (40 $\mu\text{g/ml}$) was added to a portion of a strongly inhibitory urine, and the following reaction mixtures were prepared:

A. 1.0 ml of urine containing 40 $\mu\text{g/ml}$ UDMH, 9.0 ml of buffer, and 1.0 ml of 0.5% TPF.

B. 1.0 ml of urine containing 40 $\mu\text{g/ml}$ UDMH, 8.0 ml of buffer, and 1.0 ml of 35% HCHO. After 30 seconds, 1.0 ml of 0.5 TPF was added.

C. 1.0 ml of a 40 $\mu\text{g/ml}$ aqueous UDMH standard, 9.0 ml of buffer, and 1.0 ml of 0.5% TPF.

D. 1.0 ml of a 40 $\mu\text{g/ml}$ aqueous UDMH standard, 8.0 ml of buffer, and 1.0 ml of 35 % HCHO. After 30 seconds, 1.0 ml of 0.5% TPF was added.

E. 1.0 ml of urine (no UDMH), 9.0 ml of buffer, and 1.0 ml of 0.5% TPF.

F. 1.0 ml of urine (no UDMH), 8.0 ml of buffer, and 1.0 ml of HCHO. After 30 seconds, 1.0 ml of 0.5% TPF was added.

The optical densities of solutions B,D, and F were measured 15 minutes after addition of TPF against A,C, and E, respectively. The optical densities correspond in the order given, to the values shown for "Urine + UDMH", "Aqueous Standard", and "Direct TPF Reaction" in table 4. The value 0.057 found for the "direct reaction" indicates that HCHO destroys the urine constituent that produces optical density at 500 mμ with TPF. In practice this "direct reaction" value, which is equivalent to 4.2 μg/ml of UDMH, would not be available, and the analytical results would be in error by 4.2 μg/ml. Therefore, this method of determining UDMH is not usable.

TABLE 4
EFFECT OF FORMALDEHYDE

<u>Sample</u>	<u>Optical density at 500 mμ</u>
Urine + UDMH	0.560
Aqueous Standard	0.538
Direct TPF Reaction	0.057

b. Iodine

Preliminary experiments showed that UDMH in aqueous solution was very readily oxidized by low concentrations of I₂. All the urine samples examined also reacted with iodine, so it was necessary to add considerably more iodine when urine was present than would be required by the UDMH. Usually, 1.0 ml of 1N I₂ was used. It was also necessary to remove any unreacted I₂ prior to adding TPF. This removal was accomplished by extracting with chloroform.

UDMH (40 μg/ml) was added to a portion of an inhibitory urine and also to distilled water. For convenience, enough buffer and water was added to 1.0 ml of sample to give 12.0 ml of solution to be extracted, and 10.0 ml of the extracted solution was used for color development using 0.5% TPF and a 15-minute reaction time. A reference standard was also run using the same dilutions, but without iodine treatment or extraction. The results are given in table 5.

TABLE 5
EFFECT OF IODINE ON URINARY UDMH DETERMINATIONS

<u>No.</u>	<u>Sample composition</u>	<u>Blank composition</u>	<u>Optical density, 500 mμ sample vs. blank</u>
A	UDMH, H ₂ O, buffer, TPF	H ₂ O, Buffer, TPF	0.466
B	UDMH, H ₂ O buffer, TPF	UDMH, I ₂ , H ₂ O, buffer TPF	0.477
C	UDMH, Urine, buffer, TPF	UDMH, Urine, I ₂ , buffer, TPF	0.434
D	UDMH, Urine, I ₂ , buffer	Urine, UDMH buffer	0.048
E	Urine, I ₂ , buffer, TPF	Urine, I ₂ , buffer	0.057
F	Urine, buffer, TPF	Urine, buffer	0.057

The data indicated: (1) I_2 did not significantly oxidize the urine constituent that gave optical density at 500 m μ with TPF (compare lines E and F), (2) the reaction of I_2 and UDMH did not produce optical density at 500 m μ (the optical density of line B was not smaller than that of line A), and (3) some component of the urine was oxidized by I_2 to a product which absorbed light at 500 m μ (line D).

To obtain a value for the UDMH-TPF reaction alone, it was necessary to add the values from lines C and D. This conclusion arose from consideration of the causes of absorption of light in the various solutions. The materials which could be responsible for absorption of light are:

Product of the reaction between UDMH and TPF, designated U
Product of the reaction between urine and TPF, designated V
Excess TPF, designated W
Unreactive urine constituents, designated X
Product of the reaction between urine and I_2 , designated Y

The components which would be present in the solutions of interest are:

Sample C: $U+V+W+X$
Blank C: $V+W+X+Y$
Net optical density $C = \text{Sample C} - \text{Blank C} =$
 $(U+V+W+X) - (V+W+X+Y) = U - Y$
Sample D: $Y+X$
Blank D: X
Net optical density $D = \text{Sample D} - \text{Blank D} =$
 $(Y+X) - (X) = Y$
 $C+D = (U-Y)+Y = U$, the desired value

The result of this calculation, using the data of table 5, was 0.482, and comparison with the standard (line B) value, 0.477, indicated a recovery of 101%.

Although extensive testing has not been conducted, it appears that a quantitative method for UDMH in urine can be based on the oxidation of UDMH with iodine, to form a suitable blank, and the use of 0.5% TPF. The tentative procedure follows.

Place an appropriate aliquot (not more than 8 ml) of the urine sample in each of four tubes. Add 3 ml of citrate-phosphate buffer, pH 5.4 (prepared by mixing 2 volumes of 0.2728 M Na_2HPO_4 and 1 volume of 0.2284 M citric acid), and dilute with water to 11 ml in tubes 2 and 3; 12 ml in tubes 1 and 4. To tubes 2 and 3 add 1 ml of 1 M iodine solution, mix well and allow to stand for 5 minutes. Extract all four solutions with 5 ml portions of chloroform until the aqueous phase is free of I_2 . When the phases have separated, transfer 10 ml of each aqueous layer to another tube.

Add 1 ml of 0.5% TPF to new tubes 1 and 2, and add 1 ml of water to tubes 3 and 4. Read the optical density of number 3 at 500 m μ with number 4 set to zero. Call this reading A. Fifteen minutes after the addition of TPF to the other tubes read the optical density of number 1 with number 2 set to zero, and call this reading B.

Add A plus B and, from a standard curve (aqueous solution of UDMH), determine the final concentration of UDMH giving an optical density equal to A plus B. Calculate the UDMH concentration of the urine sample by the relation:

$$\text{UDMH} = (\mu\text{g/ml equivalence of A plus B}) \times \frac{12}{10} \times \frac{1}{V}$$

where V is the volume of the aliquot of urine used as a sample.

The use of an analytical method such as this, which is specific for free UDMH, in conjunction with another, less specific (p-dimethylamino-benzaldehyde) method, may produce values whose ratio is indicative of time or extent of exposure.

B. IDENTIFICATION OF METABOLITES

1. Distribution and Balance Studies

To determine which tissue was most desirable as a source for the study of UDMH metabolism, distribution studies using C^{14} -labelled UDMH were conducted. Six female Sprague-Dawley rats, weighing 200-250 grams each were given intraperitoneal (i.p.) injections of C^{14} -UDMH at a dose of 40 mg (38 μc) per kilogram of body weight. Three animals each were killed at 1/2 hour and 4 hours postinjection, and tissues were removed and weighed. Tissues studied were brain, kidney, liver, heart, blood, and urine. To obtain a material balance, the carcass remaining after removal of the tissues was included in the distribution study. The results are given in table 6.

TABLE 6

DISTRIBUTION AND MATERIAL BALANCE STUDIES ON C^{14} -UDMH IN RATS
Concentration UDMH in tissue and percent
total dose recovered

<u>Tissue</u>	<u>1/2 Hour</u>		<u>4 Hours</u>	
	<u>$\mu\text{g/g}$</u>	<u>%</u>	<u>$\mu\text{g/g}$</u>	<u>%</u>
Brain	29	0.6	20	0.4
Kidney	78	1.4	34	0.6
Liver	38	3.0	63	3.7
Heart	12	0.1	20	0.2
Blood	12	1.3	21	2.7
Urine	1415	5.7	540	18.9
Carcass	32	75.8	19	51.2
Total Percent Recovered		87.9		77.7

Although the data of table 6 refers to experiments with rats, similar studies on dogs showed essentially the same distribution pattern.

The relatively large amounts of UDMH remaining in the carcass prompted the individual study of other organs and tissues (stomach, spleen, intestine, muscle, lung, and adrenals) to determine whether or not one of these was concentrating the material. It was found that no significant concentration was effected by any tissue except urine.

Reasonably satisfactory recoveries of C^{14} activity were obtained in these studies, but the lower recoveries encountered with the longer experiments suggested that losses due to respiration might be occurring. This possibility was investigated as described in the next section.

2. Respiration Studies

The possibility of elimination of a portion of the injected UDMH via respiration, either as UDMH or as CO₂, was investigated. Two rats were injected i.p. with 40 mg (38 µc)/kg of C¹⁴-UDMH, and each was placed in a respiration chamber in a train (shown schematically in figure 2).

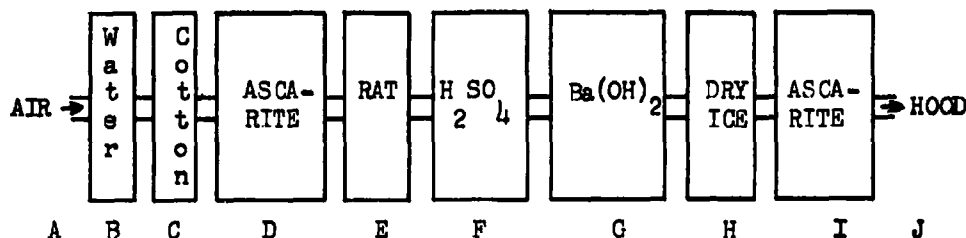


Figure 2. Respiration Train

Air from the laboratory source (A) passes through a water scrubber (B), an absorbent cotton scrubber (C) to remove moisture partially, and an Ascarite tube (D) to remove incoming CO₂ before entering the "test chamber" (E) where the rat is confined during the test. Expired gases are carried by the air stream through an acid scrubber (F) which removes basic gases, then through a saturated barium hydroxide scrubber (G) to absorb CO₂ and other acidic vapors, then through a dry-ice trap (H) to condense any neutral vapors. An Ascarite tube (I) is attached to prevent moisture from backing up into the dry-ice trap and the effluent gas is finally vented into the hood at (J). The acid, barium hydroxide, and dry-ice trap contents and the urine were analyzed for radioactivity with a liquid scintillation spectrometer.

After 4 hours the animals were removed, the receiving traps were replaced with fresh traps, and the air flow was continued for an additional 8 hours. Aliquots of all trap contents were examined for radioactivity with the results shown in table 7.

TABLE 7

CONTROL RUN FOR RESPIRATION STUDY

Sample	Total CPM		Percent of injected material	
	With rat	Without rat	With rat	Without rat
Acid Trap	14,000	30,000	0.5 ± 0.05	0.2 ± 0.02
Ba(OH) ₂ Trap	98,000	133,000	3.3 ± 0.18	4.2 ± 0.25
Dry Ice Trap				
			3.8	4.4

If respiration were the sole, or even the principal, source of gaseous C¹⁴ compounds collected in the traps, the amount collected after removal of the rat should have been quite small compared to that obtained with the rat present. The relatively high value obtained after removal suggested that most of the material collected during the rat's stay in the cage was derived from the same source as the later portion, i.e., urine as it dried in the cages. It can

be safely estimated that certainly no more than 2% (probably much less) of the injected UDMH was lost via the respiratory tract.

3. Chromatographic Examination of Tissues

All tissues used in the distribution studies (including blood and urine) were examined by paper chromatography. Aliquots (0.02-0.05 ml) of each homogenate or fluid were placed 2 1/2 inches from the end of 1" x 22 1/4" strips of Whatman No. 1 filter paper, dried, and developed 12-15 hours by the descending method with a butanol-acetic acid-water (2:1:1 by volume) solvent. After drying, duplicate chromatograms were examined by spraying with: (a) 0.5% p-dimethylaminobenzaldehyde (DAB) in a 99:1 mixture, by volume, of 95% ethanol and concentrated HCl; or (b) 0.1% aqueous solution of TFF. Other duplicates were examined for C^{14} activity.

The spray reagents rarely revealed more than two areas, but the radioactivity measurements consistently detected three major peaks in each of the tissues studied. Three lesser peaks, which may be artifacts, were occasionally encountered. None of these was present in all of the tissues. The R_f values for all six chromatographic peaks and the tissues in which they were encountered are shown in table 8. The data showed that at least three different substances were present in rat tissues following i.p. injection of UDMH.

TABLE 8
CHROMATOGRAPHIC C^{14} PEAKS

<u>Approximate R_f range</u>	<u>Status</u>	<u>Tissues in which peak appeared</u>
0.42 \pm 0.03	Definite	Urine, brain, blood, heart, liver, carcass
0.52 \pm 0.03	Probable	Liver
0.63 \pm 0.03	Definite	Urine, brain, blood, heart, liver, carcass
0.73 \pm 0.03	Probable	Brain, blood, heart, liver, carcass
0.82 \pm 0.02	Definite	Urine, brain, blood, heart, liver, carcass
0.91 \pm 0.02	Probable	Blood, heart, liver, carcass

Detection of peaks in chromatograms prepared in the manner described from dog tissues was not possible because low specific activity C^{14} -UDMH was used for injection into the dogs. An approximate 30-fold concentration was necessary to achieve levels of radioactivity comparable to those obtained in the rat studies described above. Liver was selected for chromatographic comparison because it was the only tissue in the rat which ever showed all peaks shown in table 8. A 50-ml portion of dog liver homogenate was lyophilized, and the solid was redissolved as completely as possible in 2.0 ml of water. Radioactivity measurements on chromatograms of 0.02 ml of the resulting solution revealed peaks at R_f values of 0.42, 0.60, and 0.81. These values correspond well with those of the three definite peaks shown by rat tissues. The minor peaks were not detected.

Numerous chromatograms of urine from rats and dogs which had received C^{14} -UDMH injections were prepared and examined by chemical spraying and C^{14} counting techniques. Measurement of radioactivity was considered the most reliable method of study because spray reagents are either non-specific (DAB, phosphomolybdic acid, $AgNO_3$, etc) or are too specific (TFF). Reagents in the

first category would produce colors with many urine constituents which were not related to UDMH. On the other hand, TPF produces a pink color only with UDMH under the conditions used in chromatographic studies. Therefore, it would not detect metabolites on chromatograms. A typical chromatogram of rat urine examined for radioactivity is illustrated in figure 3. The peaks at Rf values 0.44, 0.63, and 0.84 were consistently encountered, while a shoulder at Rf 0.56 was sometimes present, sometimes not.

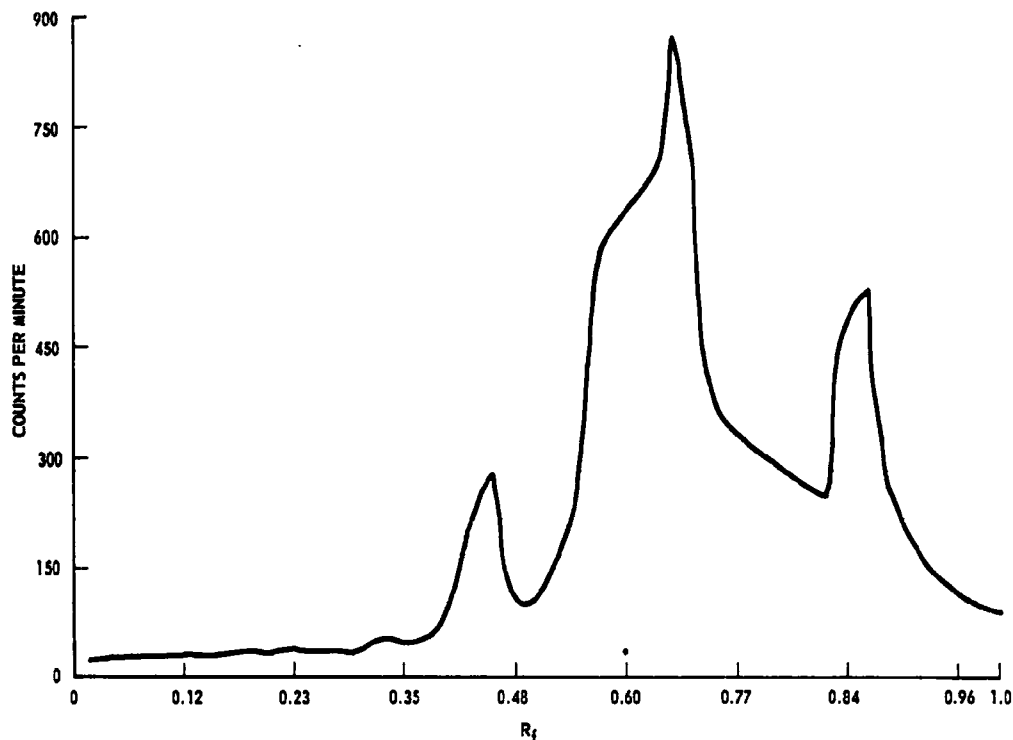


Figure 3. Chromatogram of 24-hour Rat Urine in Butanol-Acetic Acid-Water (2:1:1)

Results with spray reagents have exhibited too much variability for them to be reliable. DAB occasionally detected a substance at Rf 0.82 by the production of a purplish color rather than the yellow to orange color given by urea, amines, and hydrazines, TPF sometimes failed to detect the substance at Rf 0.63, believed to be free UDMH, even when radioactivity measurements indicated the presence of 6-10 times the quantity of UDMH required for a positive TPF reaction.

Chromatograms developed in a different solvent system exhibited a different pattern. An example of a chromatogram of rat urine developed with the solvent system butanol-ethanol -0.5 N NH_4OH (4:1:1 by volume) is presented in figure 4. The two peaks observed with this solvent appeared to correspond to the two having Rf values 0.44 and 0.84 in the previous solvent. The basis for this belief was the following study:

Chromatograms of aqueous solutions of C^{14} -UDMH were prepared using the same solvent system. Radioactivity measurements showed approximately 95% of the C^{14} was lost from the paper strips, the remainder being found at

the origin. Examination of the solvent remaining in the chromatogram tank revealed that it contained the radioactivity missing from the strips. UDMH travels with an R_f of approximately 0.6 in the butanol-acetic acid-water system, so the peak which was missing appeared to be UDMH.

These chromatographic studies established the presence of two major metabolites and UDMH in urine of rats after injection of UDMH.

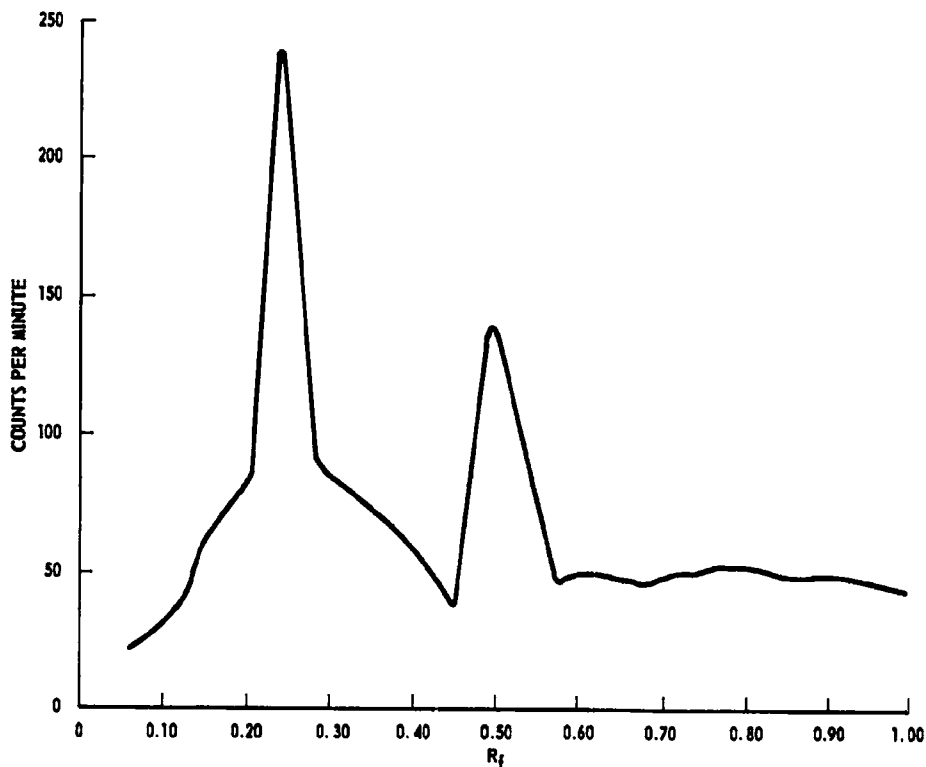


Figure 4. Chromatogram of 24-hour Rat Urine in Butanol-Ethanol-0.5N Ammonia (4:1:1)

4. Large-Scale Fractionation Studies

Attention was next directed to means of separating the metabolites found in urine in quantities sufficient for identification. The approaches considered were: (1) countercurrent extraction of urine with an organic solvent, (2) extraction of lyophilized powder from urine with an organic solvent, and (3) column chromatography on an ion exchange resin.

Preliminary experiments on liquid-liquid extractions were conducted at four different pH values (2.5, 5.0, 7.0 and 10.0) and with the solvents 1-butanol, toluene, diethyl ether, n-heptane, and carbon tetrachloride. After adjustment of the pH of portions of a 24-hour urine sample from a rat which had received an i.p. injection of 40 mg/kg C^{14} -UDMH, 0.5 ml aliquots were extracted by shaking vigorously with an equal volume of the extracting solvent. The mixtures were centrifuged to assure complete separation of phases, and aliquots of the organic layer were taken for measurement of radioactivity. Of the solvents tested only butanol extracted at least 1% of the C^{14} activity

at all pH values. Toluene extracted 2.5% of the C^{14} activity at pH 10.0, but very little at the lower pH values. Consequently, butanol was selected for more intensive study.

A 10-tube countercurrent distribution study was conducted using equal volumes of urine (24 hour collection, postinjection) and water-saturated butanol, the entire system being buffered at pH 7.4 with 0.01 M phosphate. Although the number of distributions used was insufficient for accurate measurements of distribution coefficients or for separation of metabolites, estimates of the magnitudes of the coefficients could be made. The results indicated that at least three different C^{14} -labelled materials were present. The distribution coefficients ($C_{\text{organic}}/C_{\text{aqueous}}$) of the three definitely present were 2-3, approximately 1, and less than 0.06, and the relative proportions were 1:2:5, in order of decreasing coefficients. The use of a countercurrent fractionation for separation would require either a very large number of distributions or larger portions of extracting solvent with consequent dilution. Therefore, the second extraction procedure was evaluated.

Urine from 4 rats given i.p. injections of UDMH (40 mg/kg; 38 μ g/kg) was collected for 24 hours and pooled. Portions of the pooled sample were adjusted to pH values 2.5, 5.0, 7.0, and 10.0, and several 0.5 ml aliquots of each adjusted sample were lyophilized in test tubes. Duplicates were extracted by stirring 10 minutes with 0.5 ml of the solvent being studied. The mixtures were centrifuged and radioactivity was measured in 0.1 ml of the supernatant. Water was included as one of the solvents to permit detection of losses due to volatilization or decomposition. The counting time was 1 minute for each sample and background was subtracted before equating the count rates to micrograms of UDMH. The results (table 9) are the averages of duplicate samples, and duplicates agreed within 10% except for some of those with very low count rates.

Of the solvents tested, only ethanol and butanol appeared to be of further interest, the latter appearing more promising because it extracted only part of the C^{14} activity. Paper chromatography of such butanol extracts showed that they contained most of the same C^{14} -labelled substances that are seen in tissue chromatograms, including all the major peaks (see table 8). Fractionation was not achieved, so attention was turned to ion exchange methods.

A 45-ml portion of the pooled 24-hour urine specimens from 10 experimental rats was passed through a column of 110 ml (wet volume) of Dowex 50W-X4(H+) resin. The column was washed with distilled water until C^{14} activity was no longer being eluted. The activity in the total effluent to this point (Fraction A) amounted to 8 percent of the total applied. The material was not retained by the column and its early appearance was not a result of saturating the resin with the substance. Attempts to elute the remainder of the activity from the column with NaCl, $CaCl_2$, and HCl at concentrations of 0.01-0.10 M were uniformly unsuccessful. When 0.4 M HCl was used all the residual activity was eluted, but no fractionation was achieved. A broad, badly tailing fraction was obtained, and it was not investigated further.

TABLE 9

EXTRACTION OF LYOPHILIZED URINE

UDMH concentration in extract and percent recovered

<u>Solvent</u>	<u>pH</u>							
	<u>2.5</u>		<u>5.0</u>		<u>7.0</u>		<u>10.0</u>	
	<u>µg/ml</u>	<u>%</u>	<u>µg/ml</u>	<u>%</u>	<u>µg/ml</u>	<u>%</u>	<u>µg/ml</u>	<u>%</u>
H ₂ O	170	94	170	94	168	93	72	39
95% Ethanol	170	94	170	94	170	94	39	21
Toluene	9	5	7	4	4	2	2	1
Ether	7	4	5	3	3	2	1	1
Butanol	150	83	90	49	110	60	58	32
Heptane	1	0	1	0	1	0	1	0
Carbon Tetrachloride	1	0	1	0	1	0	1	0

Fraction A was lyophilized, redissolved in 6 ml of water, and decolorized by treatment with 0.6 g of Norite A. Paper chromatograms were developed in the butanol-acetic acid-water system and examined. A large peak at R_f 0.38-0.52 was shown by both DAB and TPF sprays and a urea spot (R_f 0.60) was revealed by DAB. The latter spot was devoid of Cl^{14} activity, although the former was active.

The lack of a suitable method for separating metabolites on a scale large enough to permit the use of classical methods of identification made it necessary to use another approach. One such approach is to compare the chromatographic and/or chemical behavior of unknown metabolites with the behavior of authentic samples of suspected metabolites. Several possible metabolites were, therefore, prepared and studied as described below.

5. Synthesis of Possible Metabolitesa. Cl^{14} -labelled compoundsAcetyldimethylhydrazide

Acetic anhydride (6.0 ml, 0.064 mole) was placed in a 25 ml glass-stoppered flask and chilled in ice water 15 minutes. While swirling constantly in the bath, 3.0 ml (2.4 g, 0.04 mole) of Cl^{14} -UDMH (25 µc/ml) was added dropwise. After all the UDMH was added, a 20 µl aliquot was counted, the flask was stoppered, and the reaction mixture was allowed to stand at room temperature (26°C) 1-3/4 hours. The reaction mixture was diluted with approximately 10 ml of water and rendered slightly alkaline (pH 8.0) by the dropwise addition of 6 N NaOH.

To test the formation of the hydrazide, 0.1 ml of the alkaline solution (total volume 27.0 ml) was added to 5 ml of citrate-phosphate buffer (pH 5.4) and the pH was adjusted to 5.4 with 0.1 M HCl. Additional buffer was

added to make a total volume of 10.0 ml and the UDMH concentration was determined using 0.1% TPF. Although the aliquot used represented over 800 μ g of UDMH, the color produced with TPF corresponded to less than 5 μ g. A similar aliquot hydrolyzed by heating with alkali prior to determining UDMH produced an intense color with TPF. Apparently more than 99% of the UDMH was converted to the acetyl hydrazide.

Pyridoxal phosphate dimethylhydrazone

Five hundred mg (2.00 millimoles) of pyridoxal-5-phosphate was placed in a 16 x 150 mm test tube, and 1.0 ml of a solution containing 0.1 g (1.67 millimoles) of C^{14} -UDMH (3.1 μ c) and 0.1 g (1.67 millimoles) of acetic acid was added. The mixture, which was a semi-solid gel, was heated in boiling water for 1/2 hour. Additional hot water was added (7.0 ml) and, although much of the solid remained undissolved, the mixture was cooled in ice water and filtered. The solid was recrystallized from 50 ml of hot water (boiling water bath, temperature of mixture about 90°C), producing a yield of 313 mg (65% of theoretical based on UDMH).

An aqueous solution of the recrystallized material was prepared containing 12.96 mg (2.69 mg UDMH) in 5.0 ml of water at 50°C. One-tenth ml aliquots were used for C^{14} determination, giving a count rate of 785 ± 9 cpm, corresponding to a UDMH concentration of 0.504 mg/ml, or a purity of 93.8%.

Pyridoxal dimethylhydrazone

The procedure used for this compound was the same as that used for pyridoxal phosphate dimethylhydrazone. Greater solubility permitted the use of smaller volumes of water. Recrystallization produced brilliant yellow needles, but C^{14} counts on the product indicated that we had not obtained the expected pure compound.

Glucose dimethylhydrazone

The preparative procedure was the same as for the compounds described above, but no solid separated. During the heating period, the reaction mixture became a dark brown in color. This color may be partly, or entirely, caused by caramelization of the sugar. Attempts to isolate the derivative were unsuccessful, although a comparison of count rates and TPF analyses indicated that no more than 15.4% of the UDMH was still present as unreacted compound.

Pyruvic acid dimethylhydrazone

The same procedure was followed for this compound as for the previous hydrazones except that 0.2 ml of pyruvic acid was used. Again, isolation was not successful, although at least 74.2% of the UDMH appeared to be bound, presumably as the hydrazone.

b. Unlabelled Derivatives

Acetyldimethylhydrazide

A solution of 7.8 grams (0.1 mole) of acetyl chloride in 50 ml of diethyl ether was added dropwise to a solution of 6 grams (0.1 mole) of UDMH in 100 ml of ether. The hydrochloride of acetyldimethylhydrazide precipitated. The mixture was filtered, washed with ether, and vacuum dried

in a dry box. Nitrogen analysis: 20.4% found, 20.3% expected.

Pyruvic acid dimethylhydrazone

Three g (0.05 mole) of UDMH was added to a solution of 4.4 g (0.05 mole) of pyruvic acid in 20 ml of water. The mixture spontaneously warmed to approximately 70°, and it was allowed to stand for 1 hour, then freeze-dried. The product, a white crystalline material, was not recrystallized.

Glucose dimethylhydrazone

Six g (0.10 mole) of UDMH was added to a solution of 1.8 g (0.01 mole) of glucose in 15 ml of water containing 1 or 2 drops of glacial acetic acid as a catalyst. After standing at room temperature for an hour, the mixture was freeze-dried, producing a faintly yellow, hygroscopic solid. Nitrogen analysis: 10.0% found, 12.6% expected. No attempt at recrystallization was made.

6.06. Behavior of Synthetic Compounds

a. Chromatography

Solutions of the derivatives described above were chromatogrammed in two different solvent systems: 1-butanol:acetic acid: water = 2:1:1 and 1-butanol: ethanol: 0.5 N aqueous ammonia = 4:1:1. After development and drying, the chromatograms were sprayed with 10% aqueous phosphomolybdic acid and exposed to NH₃ vapors. This detection method will locate all types of UDMH derivatives as well as UDMH itself. The results are presented in table 10. Labelled and unlabelled products behaved alike in paper chromatography.

Comparison of the R_f values of UDMH metabolites in urine (table 8) with those exhibited by the compounds in table 10 led to the following conclusions:

(1) The largest component in urine was unaltered UDMH. This component migrated in butanol-acetic acid-water with an R_f of 0.63, as did UDMH. Furthermore, it disappeared from the paper when developed in butanol-ethanol-ammonia in the same manner as known samples of UDMH did.

TABLE 10
CHROMATOGRAPHY OF UDMH DERIVATIVES

<u>Compound</u>	<u>R_f values</u>	
	<u>Butanol-acetic acid-water</u>	<u>Butanol-ethanol ammonia</u>
Dimethylhydrazine	0.63	0.00
Acetyldimethylhydrazide	0.40	0.76
Glucose dimethylhydrazone	0.48	0.32
Pyridoxal dimethylhydrazone	0.55	0.49
Pyridoxal phosphate dimethylhydrazone	0.09	0.40
Pyruvic acid dimethylhydrazone	0.54	0.23

(2) A second component (3-10% of the total) was identified in similar fashion as glucose dimethylhydrazone. The Rf values for the metabolite in urine were 0.44 and 0.27, compared to values of 0.48 and 0.32 for a synthetic preparation.

(3) The third major metabolite in urine could not be identified with any of the compounds prepared synthetically.

b. Solvent Distribution

The acetyldimethylhydrazide solution remaining after UDMH analyses (Section 5a, above) was extracted with 7.0 ml of toluene, and a 20 μ l aliquot of each phase was counted. The count rates after subtracting background were 1,504 \pm 39 cpm for the toluene and 59,432 \pm 244 cpm for the aqueous layer. These data indicated that toluene was a poor extractant for acetyldimethylhydrazide.

The toluene was removed and 1.0 ml aliquots of the aqueous layer were extracted with 1.0 ml portions of diethyl ether, 1-butanol, heptane, or acetone. It was possible to use acetone as an extractant because the high concentration of sodium acetate (4.5 M) prevented miscibility. Aliquots of the organic and aqueous layers were counted and the cpm were equated to mg UDMH/ml. Duplicate extractions and counts were used throughout, and averages were calculated. Table 11 presents the results of these experiments and of the previously described toluene extraction.

TABLE 11
SOLVENT DISTRIBUTION OF ACETYLDIMETHYLHYDRAZIDE

<u>Extracting solvent</u>	<u>Concentration, mg UDMH/ml</u>		<u>Distribution coefficient Org/Aq</u>
	<u>Organic layer</u>	<u>Aqueous layer</u>	
Ether	1.38 \pm 0.02	60.44 \pm 0.60	0.1
n-Butanol	30.30 \pm 0.27	30.06 \pm 0.40	1.0
Heptane	0.11 \pm 0.01	64.18 \pm 0.64	0.0
Acetone	20.31 \pm 0.20	33.95 \pm 0.41	0.6
Toluene	19.10 \pm 0.38	70.80 \pm 0.21	0.3

Only butanol appeared to be of great value for extraction of acetyldimethylhydrazide. The compound showed a butanol-water distribution coefficient similar to one component of urine. Although this similarity might have suggested that acetyldimethylhydrazide was one of the compounds found in urine, the chromatographic data indicated it was not.

IV. DISCUSSION

The experiments reported have provided information concerning the intermediary metabolism of UDMH and have also led to the development of a tentative method for the determination of UDMH in urine.

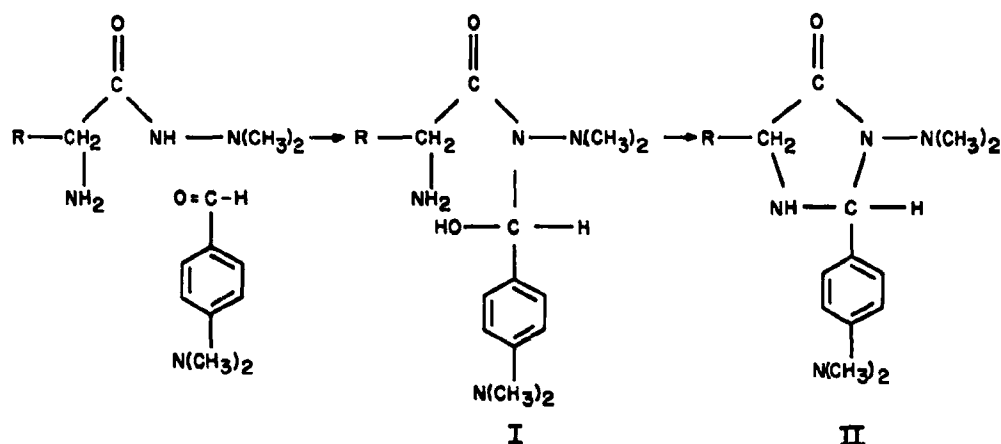
Metabolic studies have shown that a negligible amount of injected UDMH was lost via respiration. A large portion of the injected material was excreted in the urine in a few hours, and the remainder was relatively uniformly distributed in the tissues.

Matching of paper chromatographic R_f values of postinjection urine samples with that of pure UDMH in two solvent systems indicated that the major component of urine (50-60%) was unaltered UDMH. Correspondence of R_f values in two systems is considered to constitute strong evidence as to the identity of compounds. However, there were certain indications that the substance might not be free UDMH. One indication was that chromatograms sometimes failed to give a color with the TPF spray when C^{14} counting of the appropriate area indicated the presence of several times the minimum TPF-detectable quantity of UDMH. This behavior could be caused by the presence of high concentrations of a urine constituent that inhibited the TPF-UDMH reaction. In addition, when TPF reactive spots were present, the maximum color density was consistently at the leading edge of the peak of C^{14} activity. At present, this discrepancy cannot be explained.

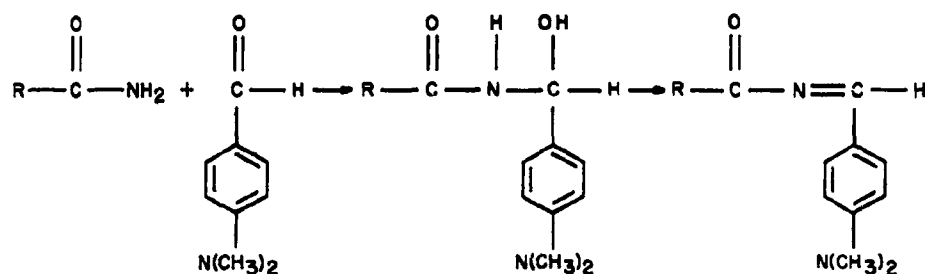
Matching of R_f data for urine with those of synthetic possible metabolites showed that the least (3-10%) of the three major components of urine was glucose dimethylhydrazone. No discrepant results were obtained regarding this metabolite.

The third major component (20-25%) of urine migrated much more rapidly than the other two in the solvent systems used. The R_f of 0.84 for this compound in the butanol-acetic acid-water system precluded its being pyridoxal dimethylhydrazone, pyridoxal phosphate dimethylhydrazone, pyruvic acid dimethylhydrazone, or acetyl dimethylhydrazide (see table 10). The relatively high rates of migration of this compound suggested that it was less water-soluble than most of the materials tested and that it was not strongly acidic or basic. If the compound were acidic or basic, its relative movement would have been decreased by the acetic acid of one system or by the ammonia of the other. The results observed indicated this compound to be a hydrazone or hydrazide of a substance whose molecular weight is greater than acetic or pyruvic acid.

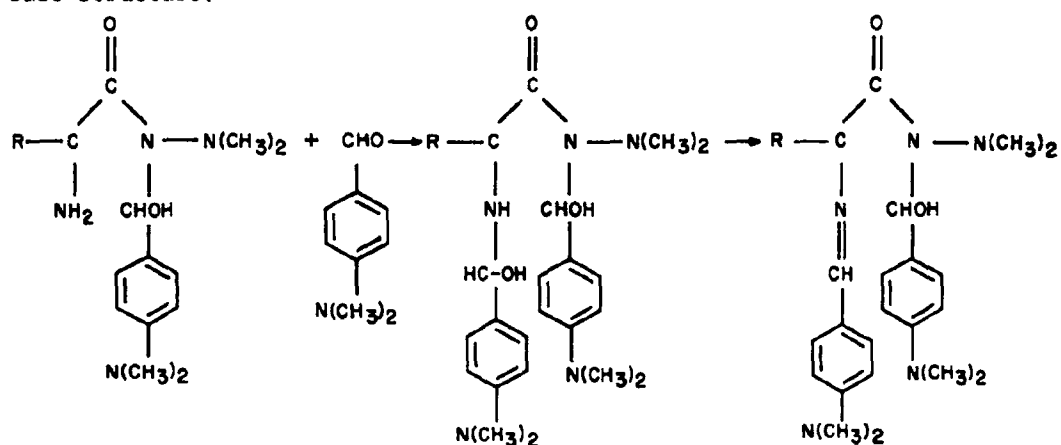
At the R_f to which this third component migrated in the butanol-acetic acid-water system, the DAB reagent occasionally produced a purplish spot. On other occasions, no DAB color reaction occurred at this R_f . The difference between this purplish color and the yellow to orange usually produced by DAB suggested that the structure of the product was fundamentally different from the orange ones or that it contained additional chromophoric or auxochromic groups. If the color-producing material is identical with the metabolite shown by radioactivity, numerous possibilities exist. For example, if the metabolite were a dimethylhydrazide of an amino acid, cyclization might occur on reaction with DAB as follows:



Compound I is a methylol derivative such as is normally formed as an intermediate in the production of Schiff bases. (Structurally, hydrazones are Schiff bases.)



However, I has no hydrogen atom remaining on the nitrogen which reacted, so dehydration must occur elsewhere. The spatial proximity of the amino group would permit its involvement in the dehydration to II, as shown. It would be equally possible that the amino group of I could react with another molecule of DAB to form a second methylol group, which could dehydrate to the Schiff base structure:



These are, at present, hypothetical possibilities.

V. SUMMARY

The distribution and metabolic fate of 1,1-dimethylhydrazine (UDMH) have been studied in rats and dogs following intraperitoneal injection of C^{14} -UDMH (40 mg/kg). Approximately 20% of the injected material was found in the urine after 4 hours. Paper chromatographic examination of body tissues revealed six compounds, although not all appeared in every tissue. Three major metabolic forms of UDMH were found in urine. These were investigated by distribution studies, ion exchange, and matching of paper chromatographic R_f values. One compound (3-10% of the total UDMH in urine) was identified as glucose dimethylhydrazone and another (50-60%) appeared to be unreacted UDMH. The third major component (20-25%) was not identified, although its chemical and chromatographic behavior suggested it was a neutral hydrazone or hydrazide of a higher molecular weight than pyruvic acid. Various analytical procedures were employed for the determination of UDMH in urine, and a trisodium pentacyanoaminoferroate (TPF) procedure was found to be the most specific and most sensitive method of those tested.

VI. REFERENCES

1. McKennis, Herbert, Jr., A.S. Yard, E.J. Adair, and J.H. Weatherby, "L-gamma-Glutamylhydrazine and the Metabolism of Hydrazine," Journal of Pharmacology and Experimental Therapeutics, Vol 21, pp 152-157, 1961.
2. McKennis, Herbert, Jr., J.H. Weatherby, and L.B. Witkin, "Studies on the Excretion of Hydrazine and Metabolites," Journal of Pharmacology and Experimental Therapeutics, Vol 114, pp 385-390, 1955.
3. McKennis, Herbert, Jr., A.S. Yard, J.H. Weatherby, and J.A. Hagy, "Acetylation of Hydrazine and the Formation of 1,2-Diacetylhydrazine in vivo," Journal of Pharmacology and Experimental Therapeutics, Vol 126, pp 109-116, 1959.
4. Reeves, J.L., Influence of Large Doses of Pyridoxine Hydrochloride on the Convulsigenic Activity of UDMH in Monkeys, School of Aerospace Medicine, Report No. 62-31, USAF Aerospace Medical Center, Brooks AFB, Texas, December 1961.
5. Satake, K and T. Seki, "Paper Chromatography of Hydrazides of Fatty Acids," Kagaku no Ryoiki (Journal of Japanese Chemistry), Vol 4, pp 557-561, 1950; Chemical Abstracts, Vol 45, p 4606, 1951.
6. Block R., R. LeStrange and G. Zweig, Paper Chromatography, Academic Press, New York, 1952, p 72.
7. Feigl, F., Spot Tests in Organic Analysis, Elsevier Publishing Company, Amsterdam, 1960, pp 128-130.
8. Feigl, F., Spot Tests in Organic Analysis, Elsevier Publishing Company, Amsterdam, 1960, pp 305-306.
9. Pinkerton, M.K., J.M. Lauer, P. Diamond, and A.A. Tamas, A Colorimetric Determination for 1,1-Dimethylhydrazine in Air, Water and Blood, Aeronautical Systems Division Technical Report 61-708, Wright-Patterson AFB, Ohio, December 1961.

<p>Aerospace Medical Division, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio Rpt. No. AMRL-TDR-62-110. STUDY OF THE INTERMEDIARY METABOLIC PATHWAYS OF 1,1-DIMETHYLHYDRAZINE (UDMH). Final report, Sep 62, iv + 22 pp. incl. illus., tables, 9 refs. Unclassified report</p> <p>The metabolic fate of 1,1-dimethylhydrazine (UDMH) was studied in female rats and dogs. The animals were given intraperitoneal injec- tions of C¹⁴ labelled UDMH and, after an appro- priate time, body tissues were examined by paper chromatography. Six compounds were found, although not all appeared in every tis- sue. Three major compounds (over)</p>	<p>Aerospace Medical Division, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio Rpt. No. AMRL-TDR-62-110. STUDY OF THE INTERMEDIARY METABOLIC PATHWAYS OF 1,1-DIMETHYLHYDRAZINE (UDMH). Final report, Sep 62, iv + 22 pp. incl. illus., tables, 9 refs. Unclassified report</p> <p>The metabolic fate of 1,1-dimethylhydrazine (UDMH) was studied in female rats and dogs. The animals were given intraperitoneal injec- tions of C¹⁴ labelled UDMH and, after an appro- priate time, body tissues were examined by paper chromatography. Six compounds were found, although not all appeared in every tis- sue. Three major compounds (over)</p>	<p>UNCLASSIFIED</p> <ol style="list-style-type: none"> 1. UDMH 2. Toxicology 3. Pharmacology 4. Industrial Medicine 5. Chromatography 6. Intermediary Metabo- lism 7. Radioisotopes 8. Laboratory Animals 9. Biochemistry I. AFSC Project 6302, Task 630202 II. Biomedical Labora- tory <p>UNCLASSIFIED</p>
<p>Identification showed one (3-10%) to be the glucose hydrazone of UDMH, and another appeared to be unreacted UDMH (50-60%). The structure of the third major component (20-25%) was not determined, although its chemical and chromatographic be- havior suggested that it was a hydrazone or hydrazide of a compound of higher molecular weight than acetic or pyruvic acid. Various analytical procedures were employed for the determination of UDMH in urine, and the tri- sodium pentacyanoaminoferroate (TPF) pro- cedure was found to be the most sensitive and most specific qualitative method of those tested.</p>	<p>Identification showed one (3-10%) to be the glucose hydrazone of UDMH, and another appeared to be unreacted UDMH (50-60%). The structure of the third major component (20-25%) was not determined, although its chemical and chromatographic be- havior suggested that it was a hydrazone or hydrazide of a compound of higher molecular weight than acetic or pyruvic acid. Various analytical procedures were employed for the determination of UDMH in urine, and the tri- sodium pentacyanoaminoferroate (TPF) pro- cedure was found to be the most sensitive and most specific qualitative method of those tested.</p>	<p>UNCLASSIFIED</p> <ol style="list-style-type: none"> III. Contract AF 33(616)- 8455 IV. Melpar, Inc., Falls Church, Virginia V. Mitz, M. A., Aldrich, F. L., and Vasta, B. M. VI. In ASTIA collection VII. Avail fr OTS: <p>UNCLASSIFIED</p>